AN INVESTIGATION OF THE NATURAL FLUORESCENCE OF WHEAT KERNEL PARTS AND FARINACEOUS RESIDUES OF CERTAIN INSECTS

by

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TABLE OF CONTENTS

INTRODUCTION

This investigation of natural fluorescence was concerned with wheat kernel parts and contamination due to farinaceous residues of certain insects; i.e., lesser grain borer (Rhyzopertha Dominica F.), granary weevil (Sitophilus granarius L.), and rice weevil (Sitophilus oryza L.).

The granary and rice weevils deposit their eggs within the wheat kernel and seal the injury with a gelatinous plug which cannot be detected by the human eye. Consequently the immature stages in concealment devour the inside of the wheat kernel and contaminate it with farinaceous residues of which the major portion cannot be removed in present milling processes. Hence, this is an important sanitation and financial problem to the milling and wheat storage industries.

Various specialized approaches have been made to this problem. Selective stains to detect the plug were developed by Frankenfeld (1) and Gossens (2) using visible light and Milner, et al. (3) using ultraviolet light. The wheat kernel was made translucent (4) by boiling in a 10 per cent solution of sodium hydroxide. Milling sanitation experts (5) developed a process known as a "cracking-flotation test." Also a cross sectioning technique (6) applied to the whole kernel yielded some information of insect damage under visible and ultraviolet light. Perhaps the most informative method used to date is radiographic (7, 8). An excellent summary of these methods by Milner (9) appeared in a recent publication.

This investigation was undertaken to determine if these substances emitted characteristic fluorescent bands, the wave lengths of these bands if existent, their relative intensity distributions, their extinction and maximum or resonant (if existing) frequencies of excitation. It was also necessary to test mixtures of these substances for reproducible results of their fluorescent intensities. These data would facilitate the development of equipment for the rapid and accurate determination of the percentage of contamination and infestation of wheat by the rice and granary weevils.

A medium quartz spectrograph was used for the determination of regions and intensities of fluorescence of rice weevil contamination, endosperm and the clean exterior of wheat kernel using a high pressure mercury lamp as a source of excitation.

The spectrograph of rice weevil contamination showed its fluorescence range to be approximately 4050 A. to 5000 A. with a broad maximum between 4300 A. and 4800 A. The endosperm and clean exterior of wheat gave almost identical spectrographs. Comparisons of microdensitometer traces of the intensity distributions yielded very small differences.

These bands appeared to be due to loosely bound molecules and are associated with the various excited states of the atoms forming the molecules. While the emission of these bands was so closely alike as to make their separation by simple methods impractical, it was still possible that they might exhibit selective excitation. It was for the purpose of determining whether this was so that the following work of measuring the fluorescent

intensity versus exciting wave length was undertaken.

APPARATUS

General views of the experimental apparatus are given in Plates I and II.

Sources

Two mercury vapor arc lamps were used as sources. One was a high pressure type with a strong continous background and the 2537 A. line reversed. The other was a General Electric four watt germicidal lamp which had 75 to 90 per cent of its output energy concentrated at 2537 A. While in use the lamp was placed in a ventilated light tight box of masonite. Inside the exit port of this box was a filter receptacle. The source filter used for all of these experiments was Corning 9863 seven and a half millimeters thick which transmitted 80 per cent of the ultraviolet light between 3950 to 2200 A. Around the outside of the port was a felt sleeve which made a light tight connection to a Bausch and Lomb monochromator.

Optical Systems

Different optical systems were used for the two types of samples.

In the horizontal set-up (Plate I) two quartz lens of approximately 10 centimeters focal length focussed the mercury line on the sample. A schematic sketch of the horizontal optical system is given.

EXPLANATION OF PLATE I

Photograph of Horizontal set-up.

A - Light tight box for source and filter.

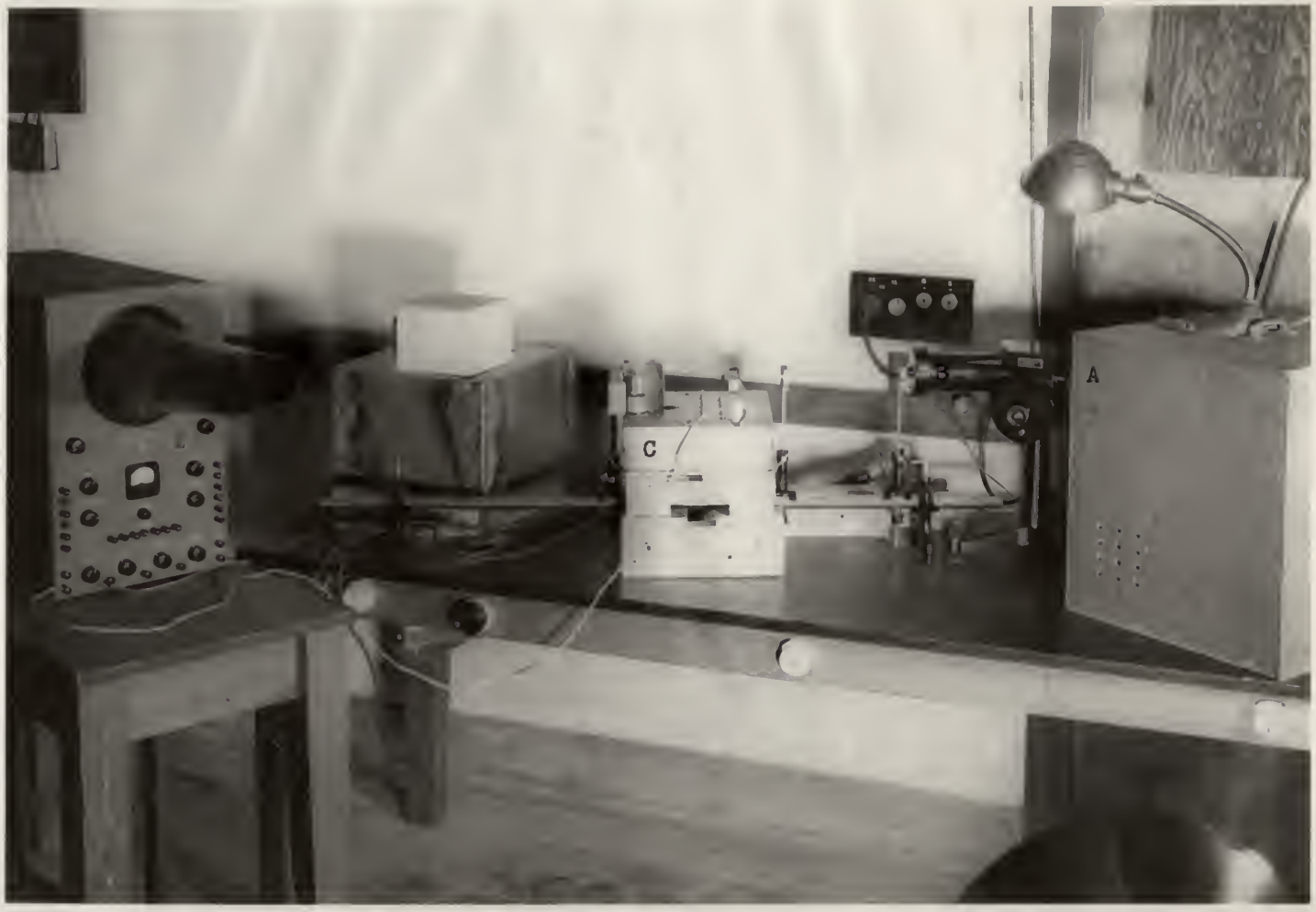
B - Bausch and Lomb monochromator.

C - Detecting unit.

D - High voltage supply.

E - D. C. oscilloscope used as an indicating unit.

PLATE I



EXPLANATION OF PLATE II

Photograph of vertical set-up.

A - Light tight box for source and filter.

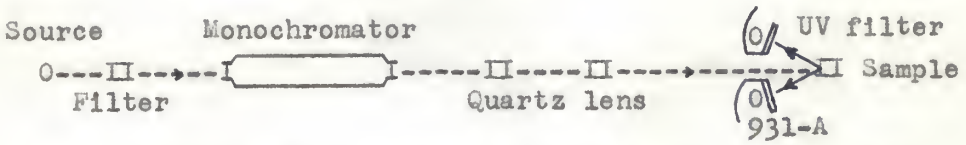
B - Bausch and Lomb monochromator.

C - Detecting unit.

D - High voltage supply.

E - D. C. oscilloscope used as an indicating device.

PLATE II



In the vertical set-up (Plate II) the first quartz lens focussed the mercury line on a one inch diameter aluminum surface mirror. This mirror changed the direction of the beam 90 degrees to downward. The second quartz lens focussed the beam from the mirror onto the sample.

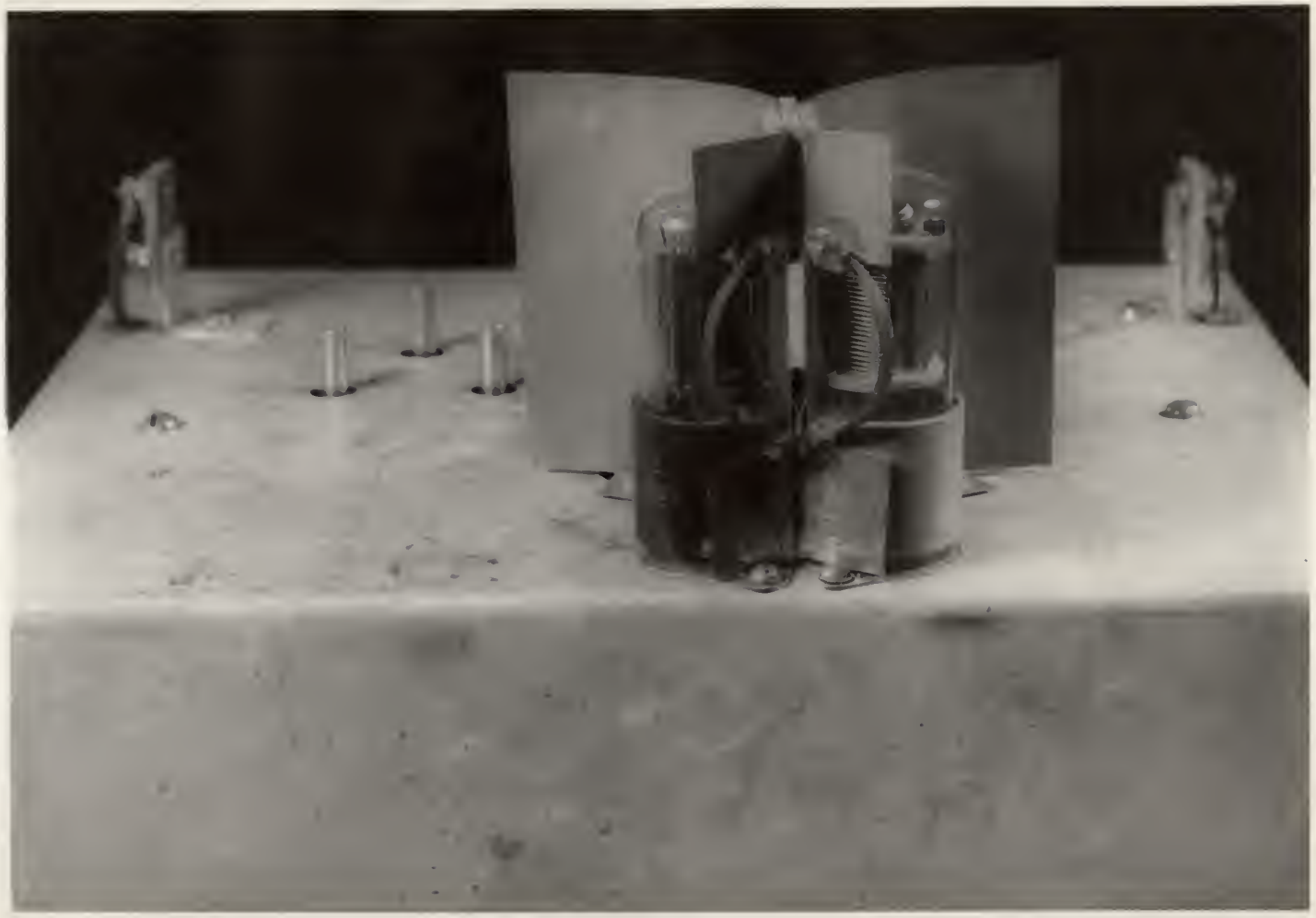
Detecting Unit

The detecting unit (Plate III) was designed for this project. Two 931-A photomultipliers were mounted on a chassis with clearance for a slit one-eighth by one and one-quarter inches between them. This slit had a depth of one inch and was formed from 18 gauge, soft copper sheet. The soft copper sheets were flared back to protect the tubes from ultraviolet radiation in the rear. Ultraviolet filters were cemented to the forward flanges parallel to the projection planes of the cathodes. These filters which cut off all wave lengths shorter than 4050A. were made from a size six Ansco UV-17 camera filter. It was cut into two equal parts along a diameter and the straight edges beveled to 30 degrees. The photomultiplier sockets were mounted with the normal line to the cathode projection planes at a 30 degree angle to the optical axis through the slit between the tubes. The distance between the cathode projection planes and the line of ultraviolet excitation on the mountings was one inch. This distance was made as short as possible to increase the subtended spherical angle.

EXPLANATION OF PLATE III

Photograph of Detecting Unit.

PLATE III



The high voltage power supply provided 1200 volts D.C. at 20 milliamperes with less than one per cent ripple. This voltage was dropped across a network of ten 22,000 ohm potentiometers paralleled with 20,000 ohm resistors. With the tubes wired in parallel approximately 95 volts per dynode stage were used with 200 volts between dynode number nine and the plate. This doubled the current sensitivity for any level of illumination and gave a voltage gain of approximately 800,000 per tube.

Since the 931-A photomultiplier tubes were operated at room temperature, a 640 micromicrofarad condenser was placed across the chassis output to reduce about 50 per cent spurious signals due to "shot effects" and thermal agitation.

Indicating Unit

An R.C.A.,D.C. oscilloscope used as an indicating device was connected to the output of the detecting unit with a shielded lead. This oscilloscope had three internal stages of D.C. amplification. The level of operation for these stages was indicated by a sensitivity meter on the front of the oscilloscope. The current output of the photomultiplier tubes passed through a voltage divider with ranges tapped off at ratios of 1:10:100:1000. The template in front of the cathode ray tube was calibrated in tenths of inches. Displacement readings in inches for each wave length were compared after they were converted to volts by multiplying the reading in inches by the sensitivity meter level and the range used.

EXPERIMENTAL PROCEDURES

After preparation of the mountings their relative intensities were measured using seven of the more intense lines in the ultraviolet spectrum of mercury. The relative intensities of these lines were also measured.

Preparation of Mountings

The base used for all mountings was a piece of one-quarter inch plywood one and three-fourths by three and one-fourth inches. On one side these were given two coats of non-fluorescing India ink for a uniform background. The respective samples were cemented to this with a cellulose acetate cement so that they covered an area in the form of a vertical line approximately one-quarter inch wide by one and a half inches long.

Seven samples were mounted. Samples of the exterior of the wheat kernel included one of the dorsal view and another of the crease view. From the interior of the wheat kernel one of the germ region cross section and another of only clean endosperm were prepared. Practically pure samples of the excrement of the rice weevil, the granary weevil, and the lesser grain borer were mounted. These samples were prepared by the tedious process of separating the insect excrement from the wheat kernel portions. Under ultraviolet light from a high pressure mercury arc lamp and a Corning filter number 9863 the excrement of the insects fluoresced an intense white compared visually to the pale blue of the wheat kernel parts.

The vertical set-up (Plate II) was used to test ground

samples of contaminated wheat for intensity of fluorescence and to reduce the time between preparation and testing. An indentation one-twelfth by one-half by one and one-fourth inches was made in these bases. After painting as before without using cement this indentation was filled level with samples to be measured.

Intensity of Fluorescence

Fluorescent intensity data were taken on the seven different mountings at each of the following wave lengths: 2537, 2652, 2799, 3021, 3131, 3341 and 3663 Angstroms. The four watt germicidal lamp was used for only the 2537 and 3663 A. lines.

After the equipment warmed up for 30 minutes the oscilloscope and monochromator were adjusted and the room blacked out. The high voltage was then applied to the indicating unit and a mounting placed in the mounting holder. All optical adjustments were checked by the displaced pattern on the oscilloscope.

Before each reading a mask was placed over the second lens, and the position of the trace on the oscilloscope was noted. This position was taken as an arbitrary zero because it compensated for any stray illumination or minor dynamic changes within the system. The pattern of the trace was sinusoidal due to the variation in intensity of the mercury arc. The recorded measurement was the maximum displacement from the arbitrary zero.

The accuracy of the oscilloscope readings had an inverse dependence on the degree of amplification used because of the increased width of the trace due to higher noise level. At

2537 A. readings were recorded to 0.10 inch and at the other wave lengths to 0.05 inch.

In tests for changes of fluorescence intensity of samples with time a standard sample was inserted before each reading as an additional check for any minor dynamic changes within the equipment.

Intensity of the Mercury Lines

The measurement of the relative intensity for the above mercury lines was accomplished using the intensity of fluorescence (10) excited in a 0.1 per cent solution of esculin, $C_{13}H_{16}O_9$, in distilled water. A two centimeter quartz cell was filled with the 0.1 per cent solution of esculin and tested for transmission of the 7 selected wave lengths by 30 minute exposures with the medium quartz spectrograph. Different wave lengths of the exciting lines penetrated to different depths of the esculin solution and the emitted fluorescent light was diminished by various amounts by partial absorption before leaving the solution. Hence to obtain true comparisons it was necessary to make measurements on the fluorescent light which had passed through a path of solution large compared with the thickness in which the exciting wave length was absorbed. With this arrangement of equipment, according to E. J. Bowen (10), the band of fluorescent light which was observed had an energy distribution within the band independent of the exciting wave length and had uniform efficiency in its production.

Using the horizontal set-up the mercury lines were focussed

on the cell in place of the sample. The detecting unit was placed on the opposite side with the photomultiplier tubes facing the quartz cell to measure the intensity of the excited fluorescence. For the wave length region 3665 to 2500 A. this arrangement acted as a relative quantum counter.

EXPERIMENTAL RESULTS

Fluorescence of Pure Samples

Six sets of data were taken of the 7 mounted samples and were averaged in volts. The 2537 A. calculations were within 5 per cent of the average while the other wave lengths were within 1 per cent of their respective averages.

The intensities of the respective mercury lines were made directly comparable by taking the most intense measurement in volts as unity and dividing the other line intensity measurements into this one. The intensity multiplying factors and their respective wave lengths with the Corning 9863 filter were: 2537 A. - 162.5, 2652 A. - 38.3, 2799 A. - 24.1, 3021 A. - 2.71, 3131 A. - 1.00, 3341 A. - 4.19 and 3663 A. - 92.8. These correction factors were then applied to the averaged oscilloscope readings in volts.

The resultant curves for the 7 mountings were directly comparable and were plotted in Figures 1 and 2. The curves confirmed much less exact visual observations.

Fluorescence of Ground Samples

The fluorescent bands were determined for the 7 different substances which in the pure state possessed very stable fluorescent intensity characteristics. It then became necessary to test

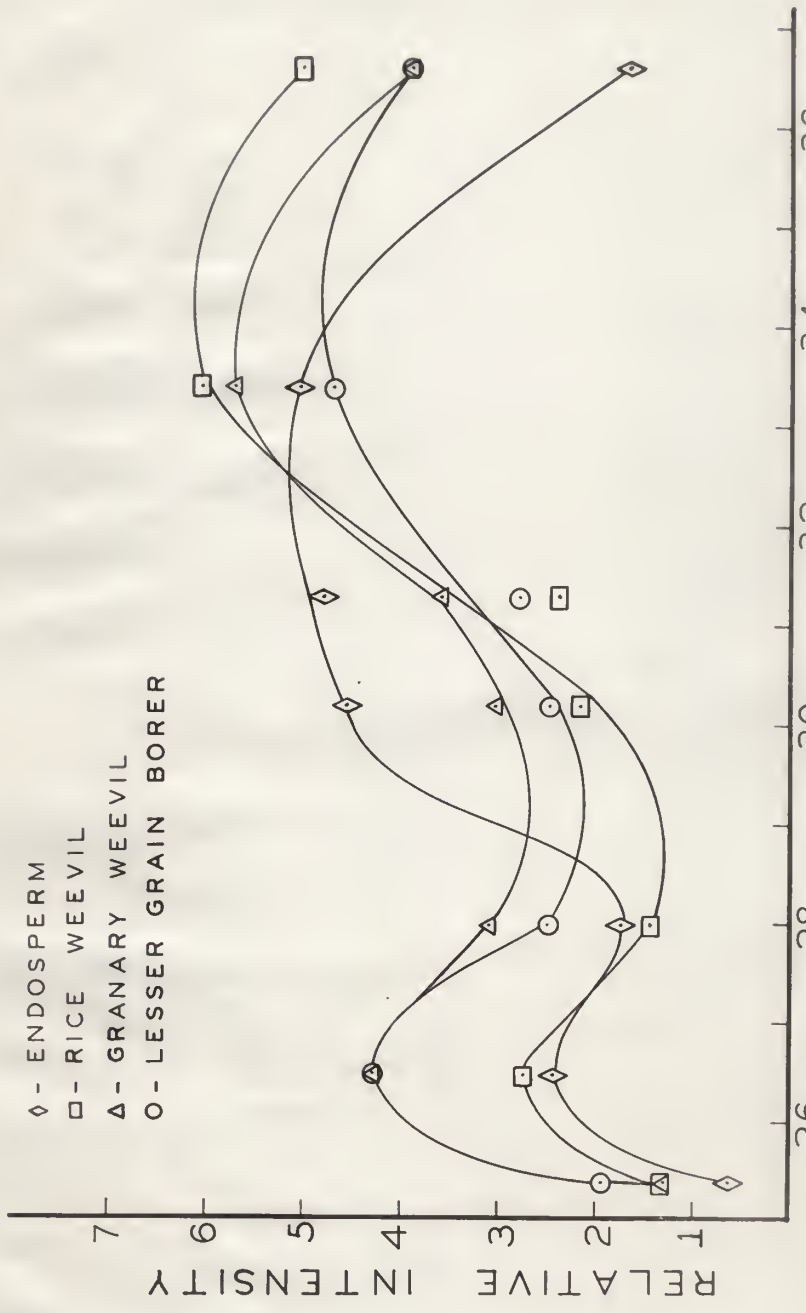


Fig. 1. Relative intensity per quanta for the pure samples.

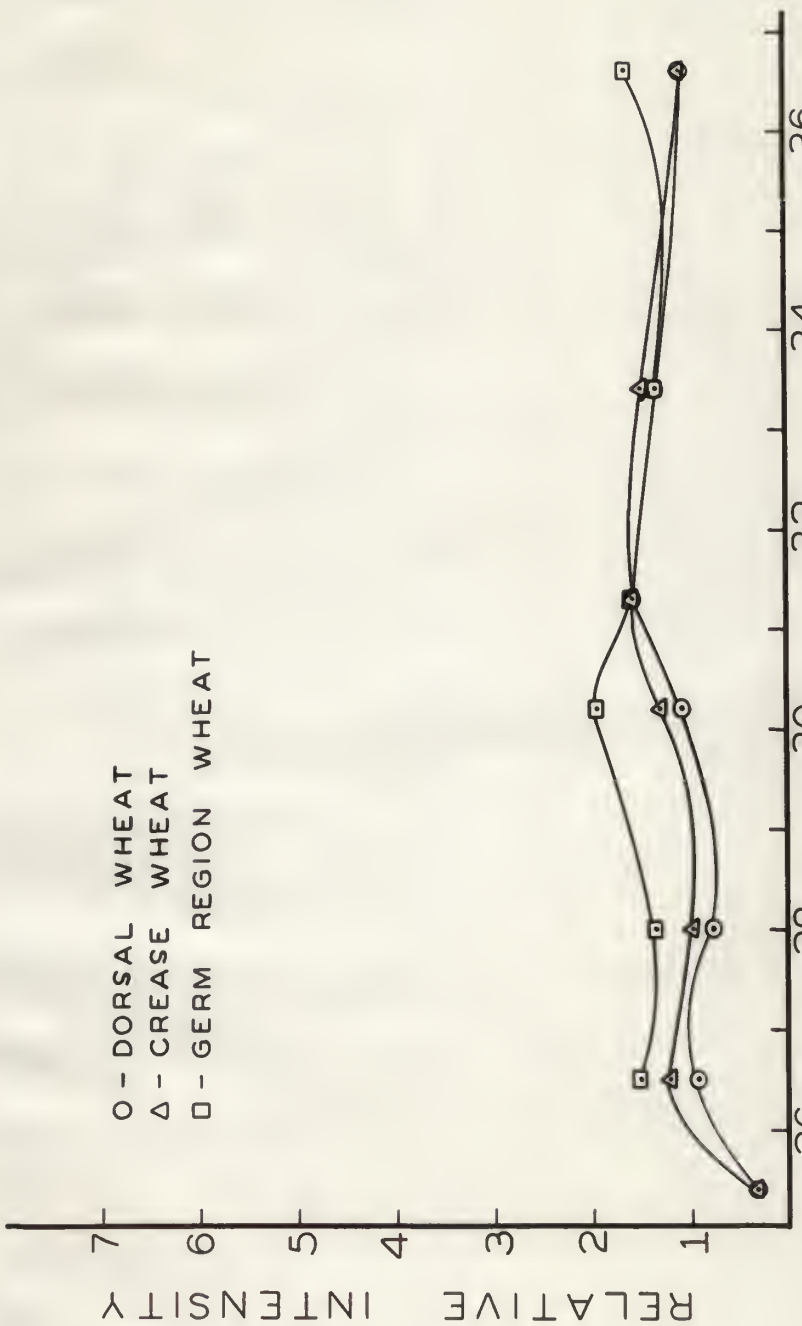


Fig. 2. Relative intensity per quanta for the pure samples.

naturally occurring mixtures of these substances for reproducible results over a period of time. These data are given in Table 1.

Table 1. The changes in per cent at the end of two hours in the fluorescent intensity for three different wave lengths of ground, screened samples of clean Pawnee wheat, rice and granary weevil 50 per cent infected wheat of the same original stock.

Wave length	Clean wheat	Rice weevil	Granary weevil
	Per Cent	Per Cent	Per Cent
3021 A.	-11.8	- 6.7	+16.7
3131 A.	- 8.0	± 6.7	+12.8
3341 A.	-23.5	-11.8	00.0

Discussion of Results

In Figure 1 the shift of the maxima to longer wave lengths of the fluorescence of the three insect residues shows similar changes in the inter- and intra-molecular structure of the principal parent substance, endosperm, in the digestive systems of these insects. The principal constituents of endosperm are gliadin, (plant gelatin) and starch. Both gliadin and starch are extremely complex substances and various compounds within each fluoresced with various colors and degrees of intensity.

According to De Ment (11) gliadin fluoresced white and starch fluoresced grey under ultraviolet excitation. The differences in the fluorescence of the insect excrements and the endosperm could probably be accounted for by reasoning that the starch underwent various degrees of decomposition in the digestive tracts of the insects while the gliadin was unaffected.

These curves show that with sufficient sensitivity to detect

small percentages of infestations there are no extinction points for the parts of the wheat kernel within practical wave length limits of an ultraviolet source for excitation.

To determine the percentage of weevil infestation before and after wheat goes into storage it was necessary that the fluorescent intensity of the exposed contaminate did not change with time. However, this did not hold as evidenced by work with ground mixtures and the changes with time were inconsistent. The decomposition of domestic animal excrements by nitrogen release processes would lead to the conclusion that comparable processes caused the time changes in the insect residues due to bacteria or enzyme actions. Also, exposure to air and light might have caused the observed time changes.

CONCLUSION

Using fluorescence as a means of measurement of the percentage of weevil infestation in wheat is impractical for the following reasons:

1. Endosperm and weevil residues fluoresce similarly both for emission and excitation.

2. Weevil and lesser grain borer (and probably other insects) residues fluoresce very similarly.

3. The magnitude of fluorescence of samples depends on time and the method of preparation.

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This investigation was to determine the fluorescent characteristics of wheat kernel parts and contamination due to farinaceous residues of the lesser grain borer, granary weevil and rice weevil. These data are necessary to ascertain whether the measurement of this fluorescence could be used for the rapid and accurate determination of the percentage of contamination and infestation of wheat by the rice and granary weevils.

Emission bands caused by excitation from the complete ultraviolet spectrum of a high pressure mercury lamp were determined with the medium quartz spectrograph. Using a Bausch and Lomb monochromator to select lines from the ultraviolet spectrum of mercury for excitation, the fluorescent intensity versus exciting wave length were measured using 931-A photomultiplier tubes and a D. C. oscilloscope.

Fluorescence is an unreliable method to determine the percentage of infestation or contamination of wheat by weevils because the fluorescent intensity from ground, naturally occurring mixtures of the above substances changes with time.